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# Telomerase Activity in Colorectal Cancer and Its Relationship to bcl-2 Expression

ATSUSHI IIDA, MD, AKIO YAMAGUCHI,* MD, AND KAZUO HIROSE, MD
*First Department of Surgery, Fukui Medical University, Fukui, Japan*

**Background and Objectives:** Telomerase is thought to be responsible for cell immortality, and bcl-2 has been demonstrated to regulate apoptosis. Recent studies have shown a wide occurrence of telomerase activation and bcl-2 deregulation in human carcinoma cells.
**Methods:** We examined telomerase activity in tissues from 50 patients with colorectal carcinoma with a telomeric repeat amplification protocol assay. We also investigated the relationship between telomerase activity and expression of bcl-2 in 37 colorectal carcinoma specimens.
**Results:** We detected telomerase activity in 33 (66%) of 50 colorectal carcinomas, whereas no activity was detected in the adjacent noncancerous mucosa of 13 tumor specimens. There was no correlation between pathological stage and telomerase activity. Telomerase activity in the bcl-2-expressing cases was higher than that in the bcl-2-non-expressing cases.
**Conclusions:** Expression of bcl-2 may be related to telomerase activity in colorectal carcinomas.
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KEY WORDS: carcinoma; telomerase; bcl-2; p53

## INTRODUCTION

Recent genetic analyses have demonstrated the existence of various oncogenes and tumor-suppresser genes and their roles in colorectal carcinomas. Carcinoma cells have a high growth or immortality property; in addition, deregulation of apoptosis was shown to contribute to the pathogenesis of carcinomas [1,2].

Bcl-2, bax, and related family members are thought to regulate apoptosis. The *bcl*-2 gene was first identified at the breakpoint of a chromosomal translocation t(14:18) in B-cell follicular lymphoma [3]. Overexpression of bcl-2 suppresses the initiation of apoptosis in response to a number of stimuli, including wild-type p53 and anticancer drugs [4–10].

Telomerase is thought to be responsible for cell immortality. In germline cells, expression of telomerase activity maintains approximately 15 to 20 kb of telomeric repeats (hexanucleotide 5′-TTAGGG-3′) at the end of replicating chromosomes, or telomeres [11,12]. Telomeres play an important role in chromosomal structural integrity and protection against DNA damage [13]. Telomeres in somatic cells progressively shorten with each cell division. This shortening of telomeres, in the absence of telomerase, results in exit from the cell cycle and cell senescence [14,15].

Although many studies have demonstrated a role for telomerase activity, it and its related factors have not been observed in human colorectal carcinoma. Since recent studies have shown a wide occurrence of telomerase activation [16–22] and bcl-2 deregulation in human carcinoma cells, we examined telomerase activity in resected specimens of human colorectal carcinoma and its relationship to bcl-2 expression.

## MATERIALS AND METHODS

### Patients and Tissue Background

We examined telomerase activity in tissue samples from 50 patients with colorectal carcinoma operated at the First Department of Surgery, Fukui Medical University, in 1995 and 1996. Samples were 50 frozen speci-

*Correspondence to: Akio Yamaguchi, MD, First Department of Surgery, Fukui Medical University, 23-3 Shimoaizuki Matsuoka, Yoshida-gun, Fukui 910-1193, Japan. Fax No.: 81-776-61-8113.
E-mail: Akio@fmsrsa.fukui-med.ac.jp
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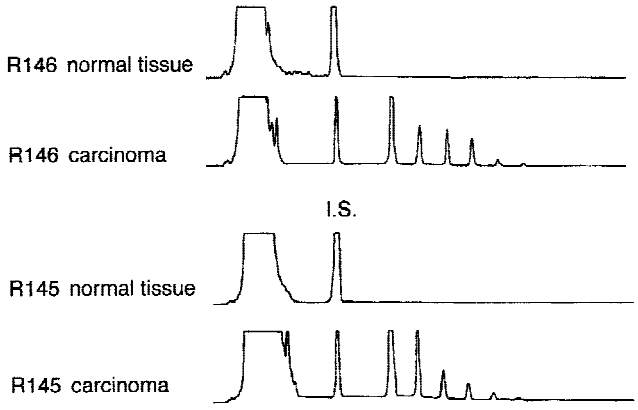


Fig. 1. Fluorocurve of telomeric repeat amplification protocol (TRAP) assay. Telomerase activity was visualized as the telomere peaks by 6 bp in the fluorocurve. I.S., internal standard.

**TABLE I. Relationship between Telomerase Activity and Clinicopathological Findings**

| Clinicopathological findings | Telomerase activity (median) | |
|---|---|---|
| Location | | |
| Colon (n = 27) | 14.8 | |
| Rectum (n = 23) | 33.6 | NS[a] |
| Wall invasions | | |
| pT1-2 (n = 6) | 21.6 | |
| pT3 (n = 28) | 23.8 | |
| pT4 (n = 16) | 29.1 | NS |
| Lymphatic invasion | | |
| − (n = 6) | 7.9 | |
| + (n = 44) | 31.6 | NS |
| Venous invasion | | |
| − (n = 21) | 28.8 | |
| + (n = 29) | 21.1 | NS |

[a]NS = not significant.

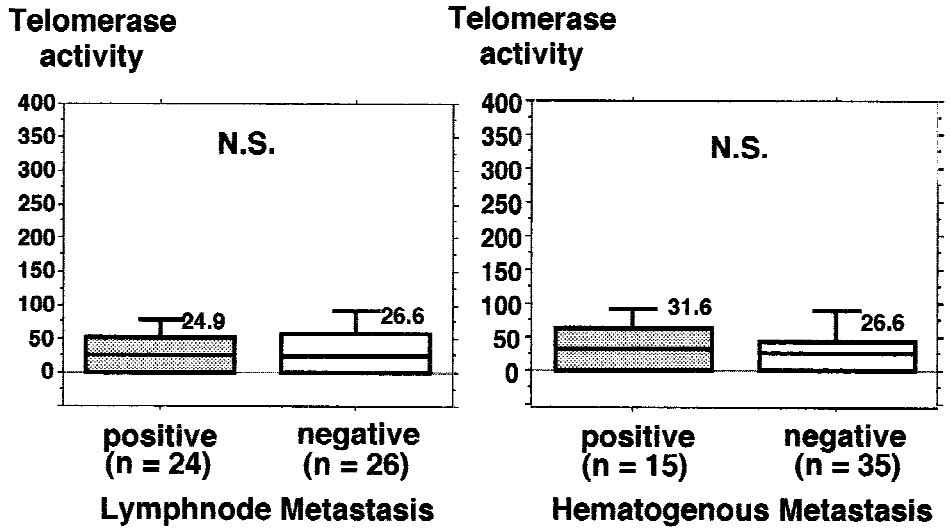


Fig. 2. Relationship between telomerase activity and lymph node or hematogenous metastasis. There was no correlation between telomerase activity and metastasis. N.S., not significant.

mens of adenocarcinomas and 13 specimens of adjacent noncancerous mucosa. All specimens were frozen within 1 h after surgical resection and stored at −80°C until use.

### Telomeric Repeat Amplification Protocol (TRAP) Assay

We homogenized the frozen samples (100 mg) in 200 ml of phosphate-buffered saline (PBS) and 3-cholamidopropyl-dimethyl-ammonio-1-propanesulfonate lysis buffer and centrifuged them at 12,000 *g* for 20 min at 4°C for protein extraction. Then, to elongate the telomeres, we incubated the extract containing 1 mg of protein with master mix for 30 min at 30°C [master mix = 10 × TRAP reaction buffer, 50 × dNTP mix, Cy-5-labeled TS primer (5′-AATCCGTCGAGCAGAGTT-3′), TRAP primer mix, Taq DNA polymerase]. After heating for 5 min at 95°C, 30 polymerase chain reaction (PCR) cycles of 94°C for 30 sec, 60°C for 30 sec, and 72°C for 45 sec were performed [16].

### Estimation of Telomerase Activity

We heated the PCR product added to formamide dye solution for 15 min at 95°C, then electrophoresed 5 ml of the PCR product on a 9% polyacrylamide gel set in an ALF Red DNA sequencer (Pharmacia Biotech, Uppsala, Sweden). Only when telomere existed in the PCR product were telomeres elongated and detected as fluorescent curves. Telomerase activity was calculated as the sum of the area in the fluorescent curve. We analyzed it by the Fragment Manager V1.1 program (Pharmacia Biotech) [23].

### Immunohistochemical Analysis

We studied expression of p53 and bcl-2 immunohistochemically in 44 patients. Sections of 44 formalin-

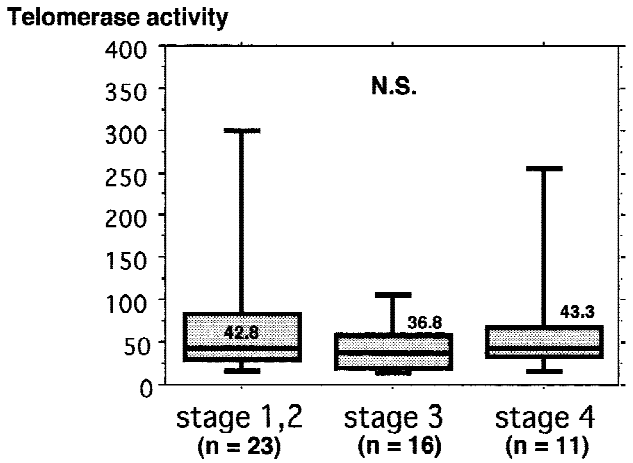


Fig. 3. There was no correlation between telomerase activity and pathological stage. N.S., not significant.

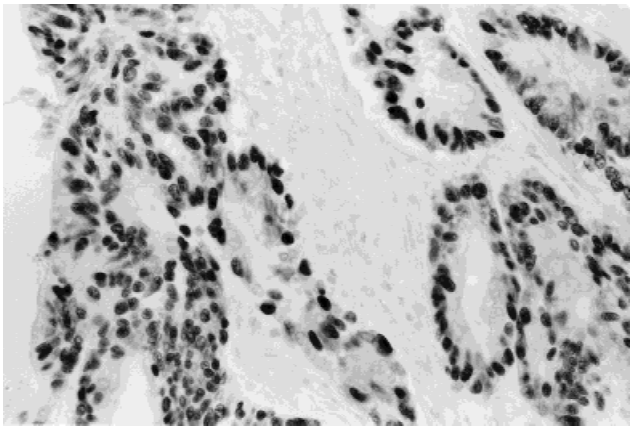

Fig. 4. High-power view of positive staining for monoclonal anti-p53 protein found on the nuclei of cancer cells (×200).

fixed and paraffin-embedded carcinomas were dewaxed and then incubated with 1% hydrogen peroxidase in methanol, to block endogenous peroxidase activity. After exposure to microwave irradiation in 10 mM citrate buffer, sections were incubated with monoclonal antibodies (anti-p53 protein, DO-1, Oncogene Science, Uniondale, NY, or antihuman bcl-2 oncoprotein, Dako, Copenhagen, Denmark) for 90 min at room temperature. We incubated sections with biotinylated goat anti-mouse immunoglobulin G (IgG) at room temperature for 30 min, then with streptavidin–biotin–peroxidase complex for 20 min at room temperature. Peroxidase activity in the sections was developed with 3-3′-diaminobenzidine tetrahydrochloride, and sections were counterstained with methyl green. We used the SW480 colon cancer cell line for positive control and replaced anti-mouse IgG antibody with monoclonal antibodies for negative control. The pathologist was blinded to the immunohistochemical results or telomerase activity for pathological diagnosis.

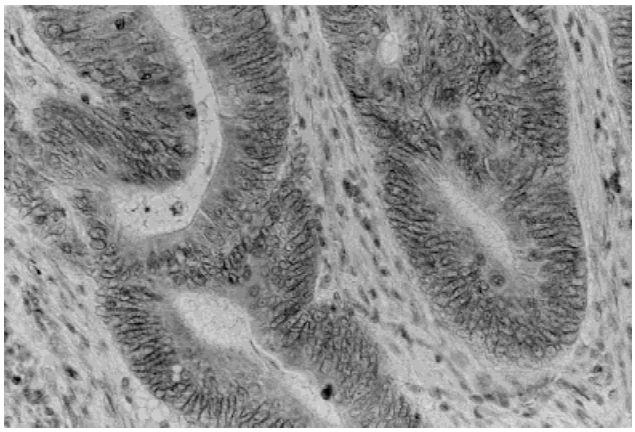

Fig. 5. High-power view of positive staining of monoclonal anti-bcl-2 protein of the cytoplasm (×200).

### Statistical Analysis

Statistical analysis was performed using the Statistical Package for Social Sciences software program (SPSS, Chicago, IL). Telomerase activities of different groups were compared by the Mann-Whitney test.

## RESULTS

Telomerase activity was visualized as the telomere peaks by 6 bp in the fluorocurve of the TRAP assay (Fig. 1). The sum of the area in their peaks revealed activities, which were compared with the 150 bp internal telomerase assay standard. We detected telomerase activity in 33 (66%) of the 50 colorectal carcinomas, whereas no activity was detected in any of the 13 samples of adjacent noncancerous mucosa.

We studied the relationship between telomerase activity and clinicopathological findings. Telomerase activity was not related to the location of tumors, wall invasion, differentiation, lymphatic invasion, or venous invasion (Table I). We also studied the correlation between telomerase activity and metastasis to lymph nodes or hematogenous metastasis (Fig. 2). No correlation between pathological stage and telomerase activity was shown (Fig. 3).

In the immunohistochemical study with monoclonal anti-p53 protein, positive staining was found on the nuclei of cancer cells in 32 (72.7%) of the 44 carcinomas (Fig. 4). However, there was no correlation between telomerase activity and p53 expression. In the study with anti-bcl-2 protein, 24 (54.5%) of the 44 carcinomas examined were stained on the cytoplasm (Fig. 5). Telomerase activity in the bcl-2-expressing cases was significantly higher than in the non-bcl-2-expressing cases (Fig. 6). These results indicate that telomerase activity may be related to bcl-2 expression in colorectal carcinomas.

## DISCUSSION

Cellular immortalization, defined as escape from physiological senescence, may be one of the major

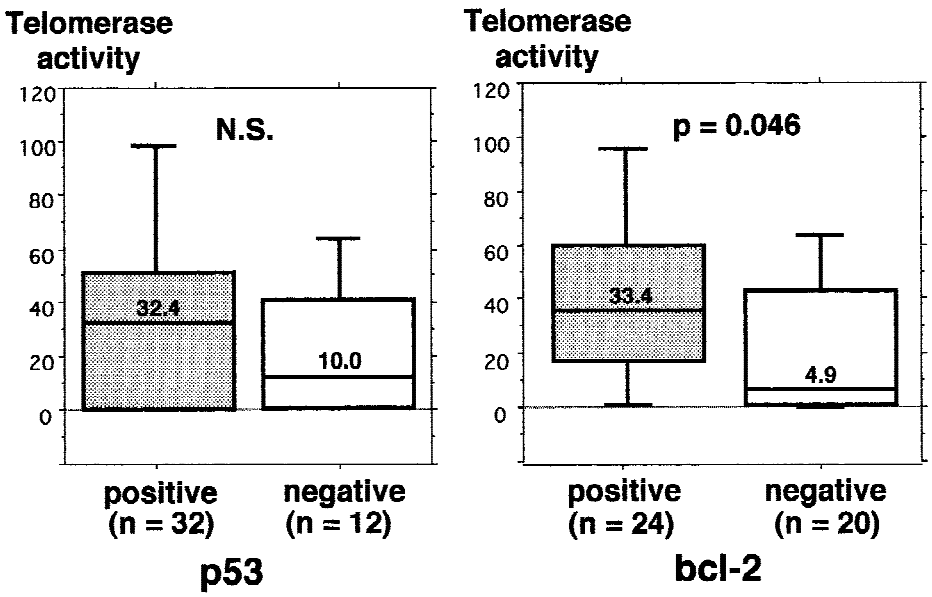


Fig. 6. Relationship between telomerase activity and p53 or bcl-2 expression. Telomerase activity in bcl-2-expressing cases was higher than in non-bcl-2-expressing cases. N.S., not significant.

events in the progression of normal cells to neoplasia. When carcinoma cells become immortal, telomeres are stabilized at a length that depends on a balance between the loss of telomeric repeats at each cycle of DNA replication and the telomeric elongation due to telomerase activity [24]. After Kim et al. [16] established the procedure to analyze telomerase activity, high frequencies of the increased activities were reported in various human tumors, such as carcinomas of the lung, breast, prostate, liver, brain, ovaries, stomach, and colon [16–22,24]. We also detected telomerase activity in 33 of the 50 colorectal carcinomas examined, and no activity was detected in the adjacent nonneoplastic mucosa. There was no relationship between the clinicopathological findings of the carcinomas and telomerase activity. These results indicate that activation of telomerase is an early event in the progression of colorectal carcinoma cells.

Overexpression of bcl-2 has been demonstrated to inhibit apoptosis in carcinoma cells [1–3]. Immunohistochemically, expression of bcl-2 protein has been detected in colorectal carcinomas and adenomas [25–28]. These findings suggest that expression of bcl-2 is also an early event in the progression of colorectal neoplasms.

The 24 cases of colorectal carcinomas with bcl-2 expression had higher telomerase activities than the 23 cases without such expression, indicating that expression of bcl-2 may be related to telomerase activity in colorectal carcinomas.

Wang et al. [7] reported that wild-type p53 inhibits bcl-2, triggering apoptosis, and Lotem and Sachs [8] reported that mutant p53 also appears to inhibit apoptosis. p53 protein has been detected immunohistochemically in colorectal carcinomas [29], but its relation to bcl-2 expression is not clear [25]. We here analyzed p53 expression in colorectal carcinomas and found no relationship with bcl-2 expression or telomerase activity. The anti-p53 protein (DO-1) may recognize a mutant p53 with an extended half-life.

The regulatory factors for telomerase activity are not clarified. We speculate that the regulatory factors for apoptosis may be key for telomerase regulation. In conclusion, telomerase may be activated as an early event of colorectal carcinoma progression. Expression of bcl-2 may be related to the telomerase activity of colorectal carcinomas.

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